A) Design and Synthesis of Nucleic Acids (oligonucleotides):
 i) Substrate Nucleic Acid

5' Specific Sequence Domain (TARGET BINDING DOMAIN)

Universal TEMPLATE
HYBRIDIZATION DOMAIN
(same for all probes)

ii) Template Nucleic Acid

3' TTTTTTTTT
SUBSTRATE

Extension Template

SUBSTRATE (SIGNAL TEMPLATE DOMAIN)
HYBRIDIZATION DOMAIN
(complementary to Template Hybridization Domain)

B) Anneal Substrate + Template Nucleic Acids in Buffer (10 minutes)

5'

C) Labeling / Extension Reaction (1 hour)
Add DNA Polymerase + α- ³²P-dATP [*A]

Labeled Substrate Nucleic Acid (PROBE)

D) Remove Unincorporated Label using Column Chromatography (10 minutes)
Optional: Remove Template and more highly purify Probe using PAGE (2 hour gel + elute)

A) Substrate and Template Nucleic Acids

Template Binding Domain

```
SEQ ID NO: 17
SEQ ID NO: 8,9,10
SEQ ID NO: 11,12,13
SEQ ID NO: 14,15,16

SEQ ID NO: 14,15,16

Substrate

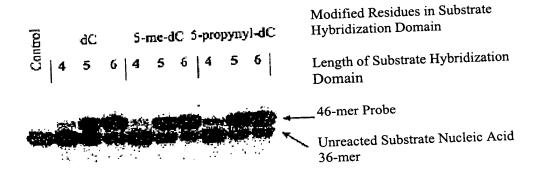
Substrate

Substrate

Substrate

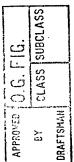
Binding Domain
```

B) PAGE analysis of reaction products



% Substrate Extended	100 80 60 40 20			The state of the s	□ DNA ■ 5Me-dC ■ pdC DNA = deoxyCytidine
	0	4	5	6	DNA = deoxyCytidine 5Me-dC = C5-Methyl-deoxyCytidine pdC = C5-Propynyl-deoxyCytidine

Length of Substrate Hybridization Domain



pGreen Lantern[™]-1 Probes

Kinase Labeled Probe

5'32P-CTTGATTAGGGTGATGGTTCACGTAGTGGGGGCGGG-3'

High Specific Activity Tailed Probe

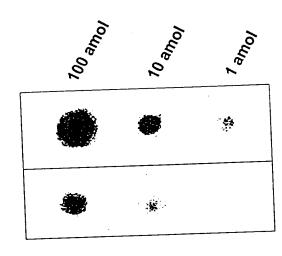
5'-CTTGATTAGGGTGATGGTTCACGTAGTGGGGGGGGGG*A*A*A*A*A*A*A*A*A*A*A

 \uparrow $* = ^{32}P \quad (10 \text{ total})$

Appanga	
27.00	C.G. 716.
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ORAFISHAH	

Dot Blot

Mass of target plasmid on Membrane



High Specific Activity Tailed Probe

Kinase Labeled Probe

FIG. 4B

E SHELLE RANGEDER

- β-Actin Probes

Kinase Labeled Probe

5' 32P-GCCCAGGAAGGAAGGCTGGAAGAGTGCCTCGGCGGG-3'

High Specific Activity Tailed Probe

5'-GCCCAGGAAGGAAGGCTGGAAGAGTGCCTCGGCGGG*A*A*A*A*A*A*A*A*A*A*A

$$\star = {}^{32}P \quad (10 \text{ total})$$

Northern Blot of Human Placental RNA

	Tailed	Kinase Labeled	Probe
28S —	- 4.		
18S —		50	—— Actin 1.9 Kb
	;÷		
	•		

Substrate Nucleic Acids and Reaction Products

SEQ ID NO: 21 DNA Substrate Nucleic Acid

5'32P-GCCCAGGAAGGAAGGCTGGAAGAGTGCCTCGGCGGG-3'

SEQ ID NO: 22 DNA Reaction Product

SEQ ID NO: 24 RNA Substrate Nucleic Acid

5' P-cugggcauggaguccuguggcauccacgaaacuaccuucaggcggg-3'

SEQ ID NO: 25 RNA Reaction Product

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PAGE analysis of Reaction Products

